Altered Pharmacological Properties of Liposome-Associated Human Interferon-Alpha†

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Human interferon-alpha was associated in different ways with positively (stearylamine) and negatively (phosphatidylserine) charged phosphatidylcholine multilamellar vesicles, depending on the presence or absence of a cholesterol component. Inclusion of cholesterol resulted in interferon that was significantly ($P = 0.0001$) more deeply internalized within the liposomes, such that detergent disruption was necessary before most of the interferon activity was expressed. Interferon was stably associated with stearylamine-containing liposomes, both with and without a cholesterol component. However, inclusion of cholesterol in the phosphatidylserine-containing liposomes was necessary for stable association of the interferon for more than 2 days at 4°C or for more than 24 h at 37°C. After intramuscular injection into mice, liposome-associated interferon in reverse-phase evaporation vesicles was retained at the local site of injection significantly longer than free interferon. Even 3 days after intramuscular injection, stearylamine-containing liposomes with or without cholesterol resulted in local interferon levels that were comparable to the peak levels obtained 2 to 4 h after free interferon was injected. In contrast, free interferon was not detectable in the local muscles 24 h after injection of $10^{4.6}$ U. Liposomes containing phosphatidylserine and cholesterol resulted in intermediate levels of local interferon retention; without a cholesterol component, phosphatidylserine-containing liposomes resulted in no increased local interferon retention compared with the results when free interferon was injected.

Liposomes have great potential as delivery systems for therapeutic agents (1, 3, 4, 6, 16). The use of liposomes can result in significantly altered bioavailability of a drug, in increased efficacy at lower doses, and in fewer unwanted side reactions (5, 10, 13, 14). Since interferon is a drug that is in short supply, has some adverse side effects, and currently requires frequent injections to have a therapeutic effect, it is a good candidate for targeted administration via a liposome delivery system.

We and others have shown recently that HuIFN- α can be incorporated successfully into REVs, SUVs (2a) and MLVs (2, 2a). We extended these studies to include a comparison of interferon capture and stability in positively and negatively charged liposomes in the presence and in the absence of cholesterol.

A major goal of incorporating interferon into liposomes is to increase its bioavailability when

it is administered *in vivo*. Liposomes alter the pharmacokinetics of drugs (8, 9, 11, 16) and after subcutaneous injection result in local retention of the encapsulated material (12, 15). Since direct intralesional injection of interferon is feasible for therapy of certain tumors, we wanted to determine whether we could increase local interferon levels by injecting liposome-associated interferon. Initially, we studied the local retention of liposome-associated interferon after it was injected intramuscularly into mice.

MATERIALS AND METHODS

Abbreviations. The following abbreviations are used in this paper: Chol, cholesterol; DOC, sodium deoxycholate; EPC, egg phosphatidylcholine; HuIFN- α , human interferon- α ; MLV, multilamellar vesicle; PS, phosphatidylserine; REV, reverse-phase evaporation vesicle; SA, stearylamine; SUV, small unilamellar vesicle; PBS, phosphate-buffered saline.

Chemicals. EPC, Chol, PS, SA, and soy bean trypsin inhibitor were obtained from Sigma Chemical Co., St. Louis, Mo.; trypsin was from Worthington Diagnostics, Freehold, N.J. HuIFN- α (3×10^6 U/mg of protein) was obtained from the Sloan-Kettering Institute Interferon Production Laboratory, Berne, Switzerland.

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Preparation of liposomes. MLVs were prepared and washed by centrifugation as previously described, using an aqueous phase containing 10^6 U of HuIFN- α per ml (2a). Briefly, the lipid film was dried in a round-bottomed flask, the aqueous interferon solution was added, and the preparation was blended with a Vortex mixer to form the MLVs. The resulting liposomes were washed in calcium-magnesium-free PBS and pelleted by centrifugation at $30,000 \times g$ for 30 min. REVs were prepared essentially as described by Szoka and Papahadjopoulos (21), except that the amount of lipid was increased to 100 $\mu\text{mol/ml}$ of aqueous phase. This modification appeared to result in slightly better capture of interferon by liposomes even though it most likely resulted in a large proportion of liposomes which had more than one lipid bilayer (F. Martin, personal communication). The aqueous phase contained 10^7 U of HuIFN- α per ml of PBS. The REVs were separated from non-encapsulated interferon by washing a 1-ml REV preparation two times in 10 ml of PBS, followed by centrifugation at $100,000 \times g$ for 30 min. Positively charged liposomes (65 mg of lipid per ml of aqueous phase) contained EPC, Chol, and SA (56.4:47:14.1, $\mu\text{mol/ml}$) or EPC and SA (78.1:19.5, $\mu\text{mol/ml}$). Negatively charged liposomes (65 mg of lipid per ml of aqueous phase) contained EPC, Chol, and PS (48:40:12, $\mu\text{mol/ml}$) or EPC and PS (67.2:16.8, $\mu\text{mol/ml}$).

Trypsin and DOC treatments. MLVs and free interferon were treated with trypsin (1.5 mg/ml), which was followed by neutralization with soy bean trypsin inhibitor (2 mg/mg of trypsin), and with 0.4% (wt/vol) DOC, as described elsewhere (2a), after incubation for 0 to 4 days at 4°C in PBS or after incubation for 1 to 72 h at 37°C either in PBS or in 50% BALB/c mouse serum. Liposomes were washed and pelleted again before trypsin treatment and DOC disruption, and all samples were diluted 1:5 (vol/vol) before they were assayed for antiviral activity.

Interferon assays. Interferon activity was determined by a microtiter assay for inhibition of cytopathic effects against vesicular stomatitis virus (19), using both human trisomy 21 cells (GM-2767; Mammalian Mutant Cell Repository, Camden, N.J.) and bovine MDBK cells.

Titers are reported as international reference units, based on National Institutes of Health human-alpha (leukocyte) interferon reference reagent G002-904-511.

Mice. BALB/c mice (6 to 8 weeks old) were obtained from Charles River Farms, North Wilmington, Mass. These mice were injected intramuscularly in both

thighs with 0.05-ml portions of the REV-interferon preparations ($10^{4.6}$ to $10^{4.9}$ interferon units per mouse) or with free interferon ($10^{4.6}$ U/mouse). One mouse from each group was sacrificed at the indicated times, and both thigh muscles were excised and quick frozen on dry ice. The muscles were homogenized and assayed for antiviral activity as 10% (wt/vol) suspensions.

RESULTS

Capture of HuIFN- α by MLVs and REVs.

Table 1 shows the capture of HuIFN- α by the different MLVs tested. The titers obtained after DOC disruption of the liposomes, which represented the total interferon that was captured by the liposomes, were slightly higher for the Chol-containing liposomes than for the liposomes lacking Chol. However, statistically there was no significant difference in the total percentage of interferon capture between the positively and negatively charged MLVs, either with or without Chol.

In contrast, without prior DOC disruption the liposomes lacking Chol showed significantly higher ($P = 0.0001$) interferon titers than the corresponding SA- or PS-containing liposomes that also contained Chol (Table 1, PBS treatment). After trypsin treatment of these liposomes, this same trend was still apparent. Thus, even though the values for total capture of interferon (DOC titer) by the different liposomes were comparable, the mode of association of the interferon with the liposomes depended on the presence of Chol.

Table 2 shows the capture of HuIFN- α by the different REVs tested. The same trend in total interferon capture observed with MLVs was obtained with REVs, with higher capture obtained when Chol was included in the liposomes. However, the total capture of interferon by REVs (10 to 20%) was higher than the total capture obtained with MLVs (4 to 5%). This result was expected for the capture of water-soluble molecules, as REVs have a higher internal aqueous volume per micromole of phospholipid than MLVs (21).

TABLE 1. Capture of HuIFN- α by MLVs having different compositions

Treatment ^a	Interferon units/ml ($\log_{10}$) ^b				P
	EPC/Chol/SA MLVs	EPC/Chol/PS MLVs	EPC/SA MLVs	EPC/PS MLVs	
Trypsin	2.2	2.5	2.9 ^c	2.9 ^c	0.0001
PBS	2.3	3.0	3.2 ^c	3.4 ^c	0.0001
DOC	3.5 ^c	3.5 ^c	3.4 ^c	3.4 ^c	0.3126

^a Liposomes (MLVs) were treated with trypsin, PBS, or DOC within 2 h after formation.

^b Liposomes were diluted 1/6 before the assay. The titer of the initial interferon preparation was $10^{4.8}$ U/ml at this same dilution. The interferon titers are the averages of six measurements. The percentages of capture were as follows: EPC/Chol/SA and EPC/Chol/PS MLVs, 5%; EPC/SA and EPC/PS MLVs, 4%.

^c Not significantly different within treatment groups at $P = 0.05$.

TABLE 2. Capture of HuIFN- α by REVs having different compositions

Treatment ^a	Interferon units/ml ($\log_{10}$) ^b			
	ECP/Chol/SA REVs	EPC/Chol/PS REVs	EPC/SA REVs	EPC/PS REVs
Trypsin	3.4	3.9	4.1	3.9
PBS	4.6	4.8	4.8	4.9
DOC	5.1	5.1	4.8	4.8

^a Liposomes (REVs) were treated with trypsin, PBS, or DOC within 2 h after formation.

^b Liposomes were diluted 1/6 before the assay. At this dilution the titer of the initial interferon preparation was $10^{5.8}$ U/ml. Interferon titers are the averages of two measurements. The percentages of capture were as follows: EPC/Chol/SA and EPC/Chol/PS REVs, 20%; EPC/SA and EPC/PS REVs, 10%.

The same trend observed with the MLVs (i.e., relatively more "latency" of interferon in liposomes containing Chol) was obtained with the REVs; that is, measurable interferon titers increased after DOC disruption of the Chol-containing liposomes, whereas this increase in interferon activity was not obtained after disruption of the REVs lacking Chol.

Stability of liposome-associated interferon in vitro. MLVs were chosen for stability studies because of the relative rapidity with which they could be pelleted, allowing more accurate mea-

surements at earlier time points. The titer of the interferon that remained liposome associated was determined after incubation in PBS for 0 to 4 days at 4°C or after incubation for 1 to 72 h at 37°C in the presence or absence of mouse serum. A statistical analysis showed that the titers did not change with time for the EPC/Chol/PS, EPC/Chol/SA, and EPC/SA vesicles or for free interferon (the slope of the plot of interferon titer versus time was not significantly different from zero), and thus these titers were averaged over time (Fig. 1A, 2, and 3).

For the EPC/PS vesicles the titers remained stable for only 0 to 2 days at 4°C and for 1 to 24 h at 37°C; thus, only the averages of these values are shown in Fig. 1B. As Fig. 4 shows, the EPC/PS MLVs showed progressive leakage of interferon after 2 days at 4°C or after 24 h at 37°C. Essentially no leakage of interferon was detected after incubation at 4°C for 2 days, and thereafter interferon leakage of approximately 1.5%/h was obtained during continued incubation at 4°C for an additional 2 days. When these vesicles were incubated at 37°C in the presence or absence of mouse serum, there was an initial rapid loss (within the first hour) of approximately 40% of the liposome-associated interferon activity; then there was no detectable interferon leakage for the next 23 h. However, after 24 h at 37°C in

FIG. 1. Stability of interferon (IFN) associated with PS-containing liposomes. MLVs prepared in the presence of 10^6 U of HuIFN- α per ml were incubated at 4°C in PBS and at 37°C in PBS or 50% mouse serum (MS). After 0, 1, 2, 3, and 4 days at 4°C and after 1, 2, 4, 24, 48, and 72 h at 37°C, liposomes were washed and then assayed for antiviral activity after no further treatment (PBS) (cross-hatched bars) or after incubation with trypsin (striped bars) or DOC (open bars). The interferon titers shown are the averages (± 1 standard deviation) of MDBK and T-21 titers from 0 to 4 days (A) or 0 to 2 days (B) at 4°C or from 1 to 72 h (A) or 1 to 24 h (B) at 37°C in PBS or mouse serum.

FIG. 2. Stability of interferon (IFN) associated with SA-containing liposomes. For an explanation of incubation conditions and symbols, see the legend to Fig. 1. MS, Mouse serum. (A) EPC/Chol/SA MLVs; (B) EPC/SA MLVs.

FIG. 3. Stability of free interferon (IFN). For an explanation of incubation conditions and symbols, see the legend to Fig. 1. MS, Mouse serum.

the presence or absence of mouse serum, there was interferon leakage of approximately 1.7%/h for the next 48 h. Thus, for the PS-containing liposomes, the inclusion of Chol was necessary for stable association of interferon with the liposomes for more than 1 to 2 days.

Although the interferon appeared to be stably associated with the liposomes for 1 to 72 h at 37°C (1 to 24 h for EPC/PS MLVs), there was an initial rapid loss (within 1 h) of approximately 0.2 $\log_{10}$ of the liposome-associated interferon after incubation at 37°C (Fig. 1 and 2 and Table 3) (PBS titers at 4°C were significantly higher than PBS titers at 37°C). The activity of free interferon was not affected by the 37°C incubation (Fig. 3 and Table 3).

Trypsin treatment had no effect on the interferon activity associated with EPC/Chol/SA MLVs (Fig. 2A and Table 3) and caused only a slight reduction (0.1 to 0.3 $\log_{10}$) in the interferon activities associated with the other types of liposomes (Fig. 1 and 2B). However, the activity of free interferon was decreased by approximately 2.5 $\log_{10}$ after a comparable trypsin treatment (Fig. 3).

DOC disruption significantly ($P = 0.0001$) increased the expression of interferon activity associated with the EPC/Chol/PS, EPC/Chol/SA, and EPC/SA MLVs (Fig. 1A and 2 and Table 3). However, DOC treatment did not have a significant effect on the interferon activity associated with EPC/PS MLVs (Fig. 1B and

FIG. 4. Leakage of interferon (IFN) from PS-containing liposomes lacking Chol. EPC/PS MLVs were incubated in PBS (open symbols) or in mouse serum (solid symbols) for 0 to 4 days at 4°C (A) or for 1 to 72 h at 37°C (B) before they were washed and assayed for antiviral activity after no further treatment (Δ and $\blacktriangle$) or after incubation with trypsin ($\circ$ and $\bullet$) or DOC ($\square$ and $\blacksquare$). The results shown are the averages of MDBK and T-21 titers.

Table 3) or on the activity of free interferon (Fig. 3 and Table 3).

Local retention of liposome-associated interferon in vitro. The retention of interferon at the sites of intramuscular injection in mice was compared with and without incorporation of

interferon into REVs. REVs rather than MLVs were used for the in vivo experiments since REVs resulted in higher interferon capture values (10 to 20% of starting interferon [Table 2]) than MLVs having the same composition (4 to 5% capture [Table 1]). The levels of free interfer-

TABLE 3. Comparison of overall stabilities of liposome-associated interferon after various treatments

Prepn	Interferon units/ml ($\log_{10}$)					
	Solution ^a			Treatment ^b		
	PBS, 4°C	PBS, 37°C	Mouse serum, 37°C	Trypsin	PBS	DOC
EPC/Chol/PS ^c	3.3 ^d	3.0 ^e	3.0 ^e	2.8 ^d	3.0 ^d	3.5 ^d
EPC/PS ^f	3.3 ^g	3.1 ^e	3.1 ^e	3.0 ^g	3.2 ^e	3.3 ^e
EPC/Chol/SA ^c	3.0 ^g	2.8 ^e	2.8 ^e	2.2 ^e	2.3 ^e	3.5 ^d
EPC/SA ^c	3.2 ^d	3.1 ^d	3.0 ^d	2.9 ^d	3.0 ^d	3.3 ^d
Free interferon ^c	4.0 ^e	4.0 ^e	ND ^h	2.8 ^d	4.8 ^e	4.8 ^e
	4.8 ^e	4.8 ^e	4.8 ^{e,i}			

^a Values were compared by solution over treatment.

^b Values were compared by treatment over solution (PBS, 4°C; PBS, 37°C; mouse serum, 37°C).

^c Values were averaged over time (0 to 4 days at 4°C; 1 to 72 h at 37°C; ≥ 30 assay values) since the slope over time was not significantly different from zero ($P > 0.05$).

^d Significantly different at $P = 0.001$ within preparation group and either solution or treatment comparison.

^e Not statistically different at $P = 0.05$ within preparation group and either solution or treatment comparison.

^f Values were averaged over time (0 to 2 days at 4°C; 1 to 24 h at 37°C; ≥ 20 assay values).

^g Significantly different at $P \leq 0.005$ within preparation group and either solution or treatment comparison.

^h ND, Not determined due to a lack of mouse serum trypsin data.

ⁱ Values were compared for PBS and DOC treatments only.

FIG. 5. Local interferon (IFN) levels after intramuscular injection of free and liposome-associated HuIFN- α . Mice were injected and sacrificed as described in the text, and the resulting 10% (wt/vol) muscle suspensions were assayed for antiviral activity on MDBK and T-21 cells. The results shown are the averages (± 1 standard deviation) of four assays. (A) Injection of $10^{4.6}$ U of free interferon. (B) Injection of interferon associated with EPC/Chol/PS REVs ($\bullet$) ($10^{4.9}$ U), EPC/Chol/PS REVs and DOC ($\blacktriangle$), and EPC/PS REVs ($\blacktriangledown$) ($10^{4.6}$ U). (C) Injection of interferon associated with EPC/Chol/SA REVs ($\bullet$) ($10^{4.9}$ U), EPC/Chol/SA REVs and DOC ($\blacktriangle$), and EPC/SA REVs ($\blacktriangledown$) ($10^{4.6}$ U).

on at the site of injection decreased rapidly after 4 h, such that no interferon activity was detected in the muscles 24 h after injection of $10^{4.6}$ interferon units (Fig. 5A), a finding which agreed with previous reports (7).

In contrast, interferon associated with EPC/Chol/PS REVs was retained at the site of injection at initially higher levels ($P = 0.0001$ at 24 h) than free interferon (Fig. 5B). This intramuscular retention was consistent with other reports concerning the local retention of vesicles at the sites of subcutaneous injection in mice (12, 15). The EPC/PS REVs, however, did not result in significantly increased local retention of interferon.

The SA-containing liposomes were the most effective liposomes in local retention of interferon (Fig. 5C). Both with and without Chol, the SA REVs resulted in significantly higher ($P = 0.0001$) initial muscle interferon levels, as well as retention of the interferon for significantly longer times ($P = 0.0001$ at 72 h) compared with free interferon.

DISCUSSION

The inclusion of high mole percentages of Chol in liposomes increases the capture and the stability of entrapment of several aqueous compounds (14, 16, 18). Therefore, we studied the effect of Chol on liposomal capture and the resulting stability of HuIFN- α . Although inclusion of Chol in the liposomal membranes resulted in slightly higher capture values for HuIFN- α by both positively (SA-containing) and negatively (PS-containing) charged MLVs, the increase was not statistically significant. However, the presence of Chol did result in increased stability of interferon associated with PS-containing liposomes.

The inclusion of Chol had an interesting effect on the apparent latency of the liposome-associated interferon. With Chol-containing liposomes, the interferon was significantly ($P = 0.0001$) more deeply internalized within the liposomes than in the absence of Chol, either within the lipid bilayer or in the aqueous compartments, such that DOC disruption was necessary before most of the interferon activity was expressed. This was especially apparent when the EPC/Chol/SA and EPC/SA MLVs were compared (Fig. 2 and Table 1). Anderson et al. (2) obtained almost complete (99%) latency of interferon by incorporating it into EPC/Chol/dicetylphosphate (7:1:2) MLVs. These authors concluded that the interferon was incorporated into the aqueous interstices of the liposomes and did not hydrophobically interact with the lipid bilayers. From our previous studies (2a) as well as the results discussed above, it appears that

depending on the liposomal composition, interferon can be associated with liposomes in different ways. With some lipid compositions, particularly when Chol is present, the interferon is internalized more deeply within the liposomes and appears to be latent until the liposomal bilayer is disrupted. With other lipid compositions, the liposome-associated interferon is trypsin resistant; yet when it is incubated with cells, this interferon results in establishment of an antiviral state without prior disruption of the liposomes. One possible explanation is that the interferon is incorporated within the lipid bilayers of the liposomes and upon association with a cell, the interferon is transferred to the cellular receptor. However, we cannot yet rule out the possibility that a specific interaction of certain liposomes with cells (22) merely results in the release of liposome-associated interferon.

The results obtained after intramuscular injection of liposome-associated interferon look promising. Significantly longer retention of HuIFN- α at the site of injection was obtained with liposome-associated interferon than with free interferon. PS-containing liposomes had to contain Chol in order to result in increased local retention of HuIFN- α , which is consistent with the in vitro stability results. Future studies will determine whether this mode of administration of interferon results in increased efficacy after intratumor inoculations.

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LITERATURE CITED

1. Alving, C. R., E. A. Steck, W. L. Chapman, Jr., V. B. Waits, L. D. Hendricks, G. M. Swartz, Jr., and W. L. Hanson. 1980. Liposomes in leishmaniasis: therapeutic effect of antimonal drugs, 8-aminoquinolines, and tetracycline. *Life Sci.* 26:2231-2238.
2. Anderson, P., J. Vilcek, and G. Weissman. 1981. Entrapment of human leukocyte interferon in the aqueous interstices of liposomes. *Infect. Immun.* 31:1099-1103.
- 2a. Eppstein, D. A., and W. E. Stewart II. 1981. Binding and capture of human interferon-alpha by reverse evaporation vesicles, multilamellar vesicles, and small unilamellar vesicles. *J. Interferon Res.* 1:495-504.
3. Fidler, I. J. 1980. Therapy of spontaneous metastases by intravenous injection of liposomes containing lymphokines. *Science* 208:1469-1471.
4. Fidler, I. J., A. Raz, W. E. Fogler, R. Kirsh, P. Bugelski, and G. Poste. 1980. Design of liposomes to improve delivery of macrophage-augmenting agents to alveolar macrophages. *Cancer Res.* 40:4460-4466.
5. Fidler, I. J., S. Sone, W. E. Fogler, and Z. L. Barnes. 1981. Eradication of spontaneous metastases and activation of alveolar macrophages by intravenous injection of liposomes containing muramyl dipeptide. *Proc. Natl. Acad. Sci. U.S.A.* 78:1680-1684.

6. Gregoriadis, G. 1978. Liposomes in therapeutic and preventative medicine: the development of the drug-carrier concept. *Ann. N.Y. Acad. Sci.* 308:343-365.
7. Hanley, D. F., M. Wiranowska-Stewart, and W. E. Stewart II. 1979. Pharmacology of interferons. I. Pharmacologic distinctions between human leukocyte and fibroblast interferons. *Int. J. Immunopharmacol.* 1:219-226.
8. Jonah, M. M., E. A. Cerny, and Y. E. Rahman. 1975. Tissue distribution of EDTA encapsulated within liposomes of varying surface properties. *Biochim. Biophys. Acta* 401:336-348.
9. Juliano, R. L., D. Stamp, and N. McCullough. 1978. Pharmacokinetics of liposome-encapsulated antitumor drugs and implications for therapy. *Ann. N.Y. Acad. Sci.* 308:411-423.
10. Kataoka, T., and T. Kobayashi. 1978. Enhancement of chemotherapeutic effect by entrapping 1- β -D-arabinofuranosylcytosine in lipid vesicles and its mode of action. *Ann. N.Y. Acad. Sci.* 308:387-394.
11. Kimelberg, H. K., and M. L. Atchison. 1978. Effects of entrapment in liposomes on the distribution, degradation, and effectiveness of methotrexate *in vivo*. *Ann. N.Y. Acad. Sci.* 308:395-409.
12. Mauk, M. R., and R. C. Gamble. 1979. Stability of lipid vesicles in tissues of the mouse: a γ -ray perturbed angular correlation study. *Proc. Natl. Acad. Sci. U.S.A.* 76:765-769.
13. Mayhew, E., D. Papahadjopoulos, Y. M. Rustum, and D. Chandrakant. 1978. Use of liposomes for the enhancement of the cytotoxic effects of cytosine arabinoside. *Ann. N.Y. Acad. Sci.* 308:371-386.
14. Mayhew, E., Y. M. Rustum, F. Szoka, and D. Papahadjopoulos. 1979. Role of cholesterol in enhancing the antitumor activity of cytosine arabinoside entrapped in liposomes. *Cancer Treat. Rep.* 63:1923-1928.
15. McDougall, I. R., J. K. Dunnick, M. L. Goris, and J. P. Kriss. 1975. *In vivo* distribution of vesicles loaded with radiopharmaceuticals: a study of different routes of administration. *J. Nucl. Med.* 16:488-491.
16. Papahadjopoulos, D. 1979. Liposomes as drug carriers. *Annu. Rep. Med. Chem.* 14:250-260.
17. Papahadjopoulos, D., R. Fraley, and T. Heath. 1980. Optimization of liposomes as a carrier system for the intracellular delivery of drugs and macromolecules, p. 151-164. *In* B. H. Tom and H. R. Six (ed.), *Liposomes and immunobiology*. Elsevier/North-Holland Publishing Co., New York.
18. Papahadjopoulos, D., S. Nir, and S. Ohki. 1971. Permeability properties of phospholipid membranes: effect of cholesterol and temperature. *Biochim. Biophys. Acta* 266:561-583.
19. Stewart, W. E., II. 1979. The interferon system. Springer-Verlag, Vienna.
20. Szoka, F., K. Jacobson, Z. Derzko, and D. Papahadjopoulos. 1980. Fluorescence studies on the mechanism of liposome-cell interactions *in vitro*. *Biochim. Biophys. Acta* 600:1-8.
21. Szoka, F. C., and D. Papahadjopoulos. 1978. A new procedure for preparation of liposomes with large internal aqueous space and high capture, by reverse phase evaporation (REV). *Proc. Natl. Acad. Sci. U.S.A.* 75:4194-4198.
22. Van Renswoude, J., and D. Hoekstra. 1981. Cell-induced leakage of liposome contents. *Biochemistry* 20:540-546.